

Utility of Artificial Intelligence in Diagnosis of Malaria and Dengue on Yumizen H 550 Cell Counter

Running title- "Artificial intelligence in cell counter"

Dinesh A Rathod, MD¹, Dr. Nita J Ninama², Dr. Viral J Patel³

¹Pathologist, Shraddhdeep Green Cross Pathology Laboratory, Gandhinagar, Gujarat, India

²Assistant Professor, Department of Physiology, Smt N H L Municipal Medical College, Ahmedabad, India

³Associate Professor, Department of Pathology Banas Medical College and Research Institute, Palanpura, Gujarat-385001, India

¹Corresponding Author E-mail: [rathodpathology09 \[at\] gmail.com](mailto:rathodpathology09[at]gmail.com)
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Abstract: ***Introduction:** Diagnosis of malaria has always been a diagnostic challenge in endemic areas. For many centuries microscopy-based diagnosis has been a gold standard method. Despite the presence of expert microscopists, laboratory misdiagnosis of malaria is still a problem. The HORIBA Medical Haematology Analyzer, Yumizen H550, provides flags for Malaria and Dengue developed through machine learning methods. It can be used as a screening tool for Malaria and Dengue fever in endemic areas. This study aimed to evaluate the sensitivity and specificity of these flags. **Methods:** The study done in western India from July-2020 to October-2020. Total 335 patients were taken. Among them, 220 were febrile patients and 115 were non febrile patients. Among 220 febrile patients, 116 patients were P.Vivax Positive, 07 were P.Falciparum positive and 32 were Dengue serology positive. Microscopic examination was considered as the confirmatory method for Malaria. Rapid malarial antigen tests were used for additional confirmation. For dengue serology markers like NS1 antigen, IgM and IgG by ELISA was used for confirmation. **Results:** In our study, at cut off point of 0.45, Sensitivity and specificity for P.Vivax was 81.03% and 90.91% respectively. **Conclusion:** Horiba Yumizen H 550 cell counter gives useful for P.Vivax flag. P.Falciparum and dengue fever flags are not effective.*

Keywords: Cell counter, Flag, Malaria, Dengue, Artificial Intelligence

1. Introduction

In India about 2 million confirmed malaria cases and 1000 deaths are reported annually. Although 15 million cases and 20000 deaths are estimated by WHO SEARO (South East Area Regional Office). India contributes 77% of total malaria in Southeast Asia. [1] Major cause of death due to febrile illness is malaria and dengue fever. For malaria diagnosis the light microscopic blood smear examination remains the gold standard method. Malaria microscopy allows the identification of the malarial parasite, their various stages and parasitic index to monitor treatment therapy. Rapid Diagnostic Tests (RDT) are relatively simple to perform, to interpret and provide rapid results (15-30 min). Recombinase polymerase amplification (RPA) is a new mode of diagnosis with high specificity. However, it is costly and is not being used extensively. Hence, the need for a sensitive and reliable test using laboratory technology. Although automated haematology analysers have not been specifically designed for malaria diagnosis, several haematology diagnostics systems demonstrated their capability to detect malaria-related abnormalities. Various complete blood count (CBC) findings suggest possibility of Malaria fever: Lower White blood count (WBC) and platelets count, Lower Red blood cells and HB level, Lower lymphocyte count while monocyte and neutrophil count significantly higher in comparison to non-malaria infected patients.

Beckman Coulter uses Volume Conductivity Scatter (VCS) technology to quantify WBCs; it can also detect the infected hemozoin ingested WBC by the difference in volume, conductivity and scatter as this has a different scatter plot and this can be used as a screening method especially in endemic area. Malaria detection in previous studies by automation used a discriminant factor derived from differences in standard deviation (SD) of volume of lymphocytes and monocytes [2]. The Cell-Dyn instruments use laser light scatter at various angles, the so called multiple angle polarized scatter separation for WBC analysis. It used to distinguish eosinophils from neutrophils based on the light depolarizing properties of their granules but has also been found to detect hemozoin pigment containing monocytes and granulocytes. [3] This detection method lacks sensitivity for early infection, as significant hemozoin production occurs only with late stages or mature Plasmodium parasite forms. Sysmex XE [4,] XN series [5] and Mindray BC-6800 [6] detect iRBCs for malaria detection. In infected red blood cells (iRBCs), the cytoskeleton is remodelled by the Plasmodium, accompanied by changes in iRBC membrane properties. These changes can result in an increased resistance to lysis for iRBCs, especially those harbouring mature forms of the parasite (late amoeboid, schizonts, gametocytes). These incompletely lysed iRBC may then produce a spurious signal in WBC channels, as a peak on the left of WBC volume histogram or as a separate cluster. Since this process is lysis-dependent, balance alarms are often

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triggered by differences between WBC counting channels using different lysis methods. This technique lacks sensitivity for low levels of parasitemia and in early infection. [7] Recently, Sysmex has released the high range XN-30 Analyzer, embedding a 405 nm violet laser with scattering and fluorescence measurements, that has a dedicated module (for additional cost) for *Plasmodium* detection and counting with a partial lysis reagent allowing parasites to remain inside iRBCs and nucleic acid staining for labelling parasites DNA, which claims an improved detection limit of 20 parasites/ μ L [8]. This device has been evaluated for malaria detection, reporting a ROC with an AUC = 0.98. and achieving 98.7% sensitivity and 96.5% specificity when compared with microscopy. [9,10]

WHO estimated 50-100 million cases of dengue fever, among them 5,00,000 cases of DHF (dengue hemorrhagic fever) reported worldwide [11]. Dengue virus is an arbovirus transmitted by Aedes mosquitoes and exists as four serotypes, DENV-1 to 4. Dengue infection can lead into severe cases with lethal bleeding tendencies due to thrombocytopenia and in the most serious cases as severe dengue fever, dengue hemorrhagic fever and dengue shock syndrome etc. Various CBC finding which suggest more possibility of dengue fever are: Leucopenia, Thrombocytopenia, higher monocyte count, higher activated lymphocyte count. Some previous studies have defined certain discriminant factors for dengue screening, such as the one developed using Cell Population Data (CPD) on Beckman-Coulter VCS instruments [12].

New screening method with the **Machine Learning** and data mining technique has been used to provide flagging in cell counter. Recently Yumizen H 550 using this for malaria and dengue screening through various construction stages like i) Data collection, ii) Variable selection, iii) Algorhythm pattern, iv) Cross validation and v) Clinical performance assessment. No added cost or expertise require, It is also

rapid, cheap and easy to interpret. Present study aims to study utility of malaria and dengue flag in Yumizen H 550. To get new cut off of better specificity and sensitivity for malaria and dengue screening.

2. Material and Method

This retrospective study was done at medium size laboratory in western India from July-2020 to October-2020. In study population patients with febrile illness included, which divided in malaria - *P.vivax* and *P.falciparum* and dengue. Healthy non febrile individuals with normal lab investigations considered as control. Sample collection done in EDTA and clot activator plain vacuum tubes. CBC done on hematology analyser YUMIZEN H550 manufactured by HORIBA Medical, Montpellier, France. Cell counter maintained as per manufacturer instructions. IQC and EQAS performance done regularly. Malaria confirmation was done by light microscopy on thin and thick smears and RDTs for malaria antigen for *P.vivax* and *P.falsiparum*. RDTs detect pLDH (Lactate dehydrogenase) and HRP-2 (histidine rich protein2) for *P.vivax* and *P.falciparum* respectively. We used FalciVax rapid kit (Tulip diagnostics, India) for malaria antigen in our laboratory. Positive Dengue case stamped by NS 1, IgM and IgG serology test done by ELISA kit (Alere, USA)

Total 335 patients were taken in study. Among them 220 were febrile patients, whereas 115 were nonfebrile patients. Febrile patients were referred with history of fever.

We used Medcalc Software (version 19.6.1) for data analysis for deriving ROC curve, P value, Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value.

3. Results

Table 1: Distribution of study population

	Age (years)		Gender		P.vivax	P. falci parum	Dengue fever
	0-14	11	Male	Female			
Febrile patients (n=220)	0-14	11	Male	65.45% (144/220)	116	07	32
	15-19	09					
	20-35	109					
	36-55	68					
	>56	23					
Non-Febrile patients (n=115)	0-14	02	Male	45.21% (52/115)	00	00	00
	15-19	00					
	20-35	38					
	36-55	53					
	>56	22					

Table 2: Performance of flag

	Mean Flag value P.Vivax (n=116)	Mean Flag value P.Falciparum (n=07)	Mean Flag value Dengue (n=32)
Febrile (n=220)	0.68	0.22	0.17
Non- febrile (n=115)	-	-	-

The P value for mean flag is <0.0001 (comparison of mean test for *P.vivax* and *Falciparum*). The P value for mean flag is 0.6965 (comparison of mean t test for Dengue and *P.Falciparum*). The P value for mean flag is <0.0001 (comparison of mean t test for *P.Vivax* and Dengue).

Table 3: ROC analysis of Malaria, Dengue flag

	Cut off	Sensitivity	Specificity	PPV	NPV
P.Vivax	0.32	83.62	24.24	1.10	0.68
	0.45	81.03	90.91	8.91	0.21
	0.68	59.48	96.97	19.63	0.42
P.Falciparum	0.21	71.43	100.00	-	0.29

	0.51	71.43	80.00	3.57	0.36
	0.77	100.00	0.00	1.00	-
Dengue	0.51	71.87	89.29	6.71	0.32
	0.60	87.50	32.14	1.29	0.39
	0.69	100.00	8.93	1.10	0.00

In our study, total 335 patients' malaria and dengue flags data were taken. Among 335 total patients, 220 were febrile patients and 115 were nonfebrile patients. Among 220 febrile patients, 116 patients were found malaria P.vivax positive patients, 07 were P.falsiparum positive patients and 32 were dengue positive patients.

Among total 335 patients, 149 patients showed flag for malaria 'P.Vivax' in various range of flag. 116 patients were found true positives which were all febrile patients. 33 were false positives. Among total 335 patients, 13 patients showed flag for malaria 'P.Falciparum' in various range of flag. Only 3 patients were found true positives which were all febrile patients. Other 4 patients were false negatives, who didn't have flag for p. falciparum, but they were positive by RDTs. Among total 335 patients, 65 patients showed flag for malaria 'dengue' in various range of flag. Only 9 patients were found true positives which were all febrile patients. Other 23 patients were false negatives, who didn't have flag for Dengue, but they were positive by RDTs. All other remaining patients were true negatives.

4. Discussion

Cell counter AI based Malaria and dengue disease flag value can be very useful in Indian scenario as add on of test like thick smear, rapid antigen which increase time and cost and require expert input. High end haematology analyser machines are not available everywhere. With use of low to medium range HORIBA YUMIZEN H550 haematology analyser, we help our patients with early malaria and dengue screening. In Yumizen H550 artificial intelligence (AI) uses WBC scatter analysis involving lyse resistant RBC to derive malaria and dengue flag. There are many variable AI uses to derive value like total WBC count, manufacturer has did not disclose it, however this flag are only for screening, not definite flag. In our study we found that significant difference in flag value of P .Vivax and P.Falciparum ($P < 0.0001$). However, there is no significance difference found in flag value with low versus high parasitic load ($P = 0.0051$).

For malaria P.vivax, in our study at cut-off point of 0.45, sensitivity was 81.03% and specificity was 90.91%. In Dharap et al area under the ROC curve was 0.9 for P.vivax. At cut off point off 0.5, sensitivity was 65.2% and specificity was 98.2%. Our Data are comparable with Dharap et al study [13]. From above value we could define that 0.45 cut off value would be ideal for malaria screening. Machine flagging is reliable for malaria P.vivax.

In malaria falciparum flag, true positive rate is low. However due to very low number of patient ($n=3$) we cannot derive statistical significant value hence not in discussion. In Dengue, only 9 patients were found true positives. In Dharap et al study, area under the ROC curve was 0.904 for Dengue. At cut off point off 0.5, sensitivity was 79.3% and specificity

was 86.4% [13]. In our study true positivity of dengue is less may be due to AI is dynamic process which involve multiple event and CBC parameter algorithm results to give predictive value. So, specific value for any viral disease is not reliable. As AI needs higher data input to become more robust, the manufacturer should gather all raw cell counter data and include in master for AI flag, so flag can improve year by year. The different model of same cell counter behaves differently in AI, how cell counter is maintained is also important to derive proper validate data output.

5. Conclusion

Yumizen H 550 Flagging for malaria P.vivax is reliable. We can use them as screening test. 0.45 is the best cut off point for malaria P.Vivax screening.

For dengue flag is found to be non-specific. So better we can call it "Viral Flag" rather than specify it as dengue flag. More data update and input required by manufacture for making flag more specific.

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Performance evaluation of machine learning-based infectious screening flags on the HORIBA Medical Yumizen H550 Haematology Analyzer for vivax malaria and dengue fever.